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TITLE: TRANSLATIONAL ADVANCEMENT OF SOMATOSTATIN GENE DELIVERY FOR DISEASE MODIFICATION AND COGNITIVE SPARING IN INTRACTABLE EPILEPSY

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14. ABSTRACT

Roughly a third of epileptic patients cannot be satisfactorily treated by medication or surgery, and delayed development of epil epsy is a frequent outcome from brain trauma. Intracranial gene transfer could provide an alternative to surgical resection in p harmacologically resistant epilepsy, or where resection is not an option. Thisproject tests whether somatostatin, delivered usin g a gene therapy approach, can modify epileptic phenotypes in a ratkindling model of epilepsy by modulating aberrant hippoca mpal neuron production and neuroinflammation. To date, we have replicated our finding that the delivery of a somatostatin gen e to hippocampal neurons, using a vector based on adeno-associated virus, can reverse the most severe Class 5 seizure beha vioral phenotype. We have generated strong preliminary evidence that the seizure kindling procedure strongly and selectively upregulates somatostatin type-2 receptor protein expression on hippocampal astrocytes. Kindling also strongly upregulates th e production of new hippocampal dentate gyrus cells in these animals. We are currently quantifying these effects. We are gen erating the kindled seizure animals in which we can test the effects of somatostatin gene delivery on aberrant neurogenesis, n euroinflammation and learning and memory. The studies will establish improved understanding of therapeutic mechanisms, tr anslational suitability, and appropriate outcome measures to advance toward more effective treatment ofepilepsy.

15. SUBJECT TERMS
Epilepsy; seizure; kindling; somatatostatin; traumatic brain injury; gene delivery; adeno-associated viral vector; neurogenesis; i nflammation; neurodegeneration; hippocampus, memory

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INTRODUCTION: Roughly 30% of epileptics cannot be satisfactorily treated by medication or surgery. Brain injury significantly increases risk for epilepsy, and the high incidence of brain injuries translates into substantial numbers of new intractable epilepsy cases in military personnel. Although the neuropeptide somatostatin (SST) synthesized in neurons exhibits synaptic release and activates synaptic receptor-effector cascades, receptors on non-neuronal cells in brain, on macrophages, and peripheral interactions with classical inflammation mechanisms (TNFalpha, cytokines) implicate brain SST in inflammation and cellular proliferation controls. Both are disrupted in epilepsy and may contribute to neuropathology and functional impairment beyond seizures *per se*. Brain neurons expressing somatostatin are especially vulnerable to seizure-related loss, but somatostatin gene transfer cannot mediate the same synaptic functions so efficacy is likely to be associated with other biological actions, possible related to anti-proliferation and anti-inflammatory properties recognized for SST outside the brain. Our initial tests demonstrated that intracranial somatostatin gene delivery prevented the evolution to high-level seizures in 70% of rats treated in an established electrical brain stimulation regimen. This impressive reduction in experimental epileptogenesis may provide strategies for new effective therapy for intractable epilepsy, but the efficacy mechanisms and parameters must be established prior to informed clinical development. We predict that somatostatin gene transfer will counteract the effects of epileptic development on the proliferation rate of hippocampal progenitor cells, their long-term phenotype differentiation, and their contribution to pro-convulsive networks. Second, hippocampal somatostatin gene delivery will reduce inflammation signaling that is stimulated by seizures and probably contributes to synaptic dysfunction, cell viability, and a progression to increasingly severe seizures. A thoroughly characterized rodent epilepsy model will be used as a platform to test the hypotheses. In this model temporal lobe electrical stimulation initially does not cause seizures, but gradually the same level of stimulation causes progressively more severe seizures over days to weeks. This induction of a seizure-prone state, called kindling, is measured by behavioral and electrographic severity, the number of stimulations required to produce maximally severe seizures, and the amount of damage displayed by cells in the brain post-mortem. The progressive hyperexcitability that develops in conjunction with lowered seizure thresholds is a therapeutic disease modification target distinct from seizures *per se*. Localized gene delivery using adeno-associated viral vectors for SST or inactive control protein will be tested for ability to suppress seizure susceptibility and severity during kindling. Cellular proliferation and maturation mileposts, and brain inflammation pathway markers, will be evaluated to ascertain whether either are altered in relation to SST effects on seizure severity. If they are not altered then the efficacy of SST gene delivery against seizure susceptibility most likely depends on other mechanisms. The evolution of epilepsy between an initial insult and recurrent spontaneous seizures is the most opportune time for therapeutic intervention, because loss of important neuronal populations is likely to have already occurred when these emerge, and because seizures tend to become more severe and/or frequent once recurrent seizures begin. Somatostatin gene delivery uses vectors currently performing well in human clinical trials, and could provide a new, safe, and effective way to interfere with this evolution following brain injury.

KEYWORDS: Epilepsy; seizure; kindling; somatatostatin; traumatic brain injury; gene delivery; adeno-associated viral vector; neurogenesis; inflammation; neurodegeneration; hippocampus, memory

OVERALL PROJECT SUMMARY: Progress during the reporting year is congruent with the approved Statement of Work (SOW). Experimental designs and methods have not changed and animal use continues to advance the Specific Aims by satisfying the identified tasks. There have been no actual or anticipated delays, or changes in the objectives. The communicating PI (MK) requested and received approval for a change in effort to accommodate the terms of a Small Projects in Rehabilitation project sponsored by the Rehabilitation Research and Development Service of the Department of Veterans Affairs. This complementary project will specifically evaluate safety and efficacy of the same gene therapy but in an animal model that combines TBI and seizures.

Statement of Work
Specific Aims:
1. Does SST gene transfer alter hippocampal neurogenesis in relation to behavioral or brain pathology during development of seizures in a rat kindling model of epileptogenesis? (Tasks 1, 2, 3, 4)
2. Does SST gene transfer alter inflammation cascades over the time scale in which epileptogenesis occurs? (Tasks 1, 2, 5, 6)

Task 1. IACUC & ACURO (months 1-3)

IACUC and ACURO approval were obtained prior to the initiation of the project.

Task 2. Preparation and validation of gene transfer vectors (months 1-2)

One AAV-SST and one AAV-GFP preparation were generated by the University of Florida Vector Core. Validation is continuous by standard histological visualization of SST and GFP transgene proteins in all experimental animal brain specimens.

Task 3. Experiment 1: SST vector effects on dentate gyrus cell proliferation and integration after initial seizure (months 3-20)
Four experimental treatment groups of 10/group will be generated from rats that meet inclusion criteria after surgical implantation of indwelling stimulation and recording electrode headsets and gene transfer. These 40 rats will undergo behavioral testing before and after surgery. After exhibiting the first kindled seizure they will be labeled for proliferating cells, and 1 day later the brains will be harvested for anatomical analyses of multiple cell phenotype markers. Behavioral, seizure, and anatomical data will be analysed, interpreted, and written for peer-reviewed publication. The design and time constraints require sequential testing of small numbers of rats at a time, so that new animals will be generated continuously until the treatment groups have been filled. Completion of the described subtasks for 40 rats is estimated to require 68 weeks (17 months).
3a. Presurgical alternation behavior (months 3-19)
3b. Surgery for gene delivery, kindling electrode stimulation and recording electrode placement (months 3-19)
3c. Post-surgical alternation behavior (months 3-19)
3d. Pre-kindling BrdU administration (months 3-19)
3e. Kindling (months 3-19)
3f. Euthanasia and histological processing (months 4-20)
3g. Histometry (months 4-20)
3h. Statistical analysis (months 18-20)
3i. Manuscript preparation and review (months 20-21)

Task 5: Experiment 3: SST vector effects on dentate gyrus inflammation after initial seizure (months 3-20)
Another 4 experimental treatment groups of 10/group will be generated from rats that meet inclusion criteria after surgical implantation of indwelling stimulation and recording electrode headsets and gene transfer. These 40 rats will undergo behavioral testing before and after surgery. A day after exhibiting the first kindled seizure they will be euthanized with brains harvested for of multiple inflammation signaling markers. Parallel biochemical (BioPlex) and anatomical analyses will be conducted. Behavioral, seizure, biochemical, and anatomical data will be analysed, interpreted, and written for peer-reviewed publication. The design and time constraints require sequential testing of small numbers of rats at a time, so that new animals will be generated continuously until the treatment groups have been filled. Completion of the described subtasks for 40 rats is estimated to require 68 weeks (17 months) and can run in parallel with Task 3.

5a. Presurgical alternation behavior (months 3-19)
5b. Surgery for gene delivery, kindling electrode stimulation and recording electrode placement (months 3-19)
5c. Post-surgical alternation behavior (months 3-19)
5d. Pre-kindling BrdU administration (months 3-19)
5e. Kindling (months 3-19)
5f. Euthanasia and BioPlex processing (months 4-20)
5g. Histometry (months 4-20)
5h. Statistical analysis (months 18-20)
5i. Manuscript preparation and review (months 20-21)

Tasks 4 and 6 are not scheduled to begin until month 18, so are omitted here.

After receiving her Master's degree in Dr. Carney's lab, Gowri Natarajan, became a doctoral program student supported by CoPI Carney subcontract. She has been integral to the project and highly productive throughout the reporting year. Ms. Natarajan completed formal laboratory rotations in the Carney and King laboratories, and more recently has been trained in the Ormerod lab to perform confocal microscopy, neurogenesis histology, and BrDU injections. This summer she completed her doctoral coursework, distinguishing herself at the top of her class in medical neuroscience and advancing to candidacy for the Ph.D. She has generated all of the kindled animals as well as performed all vector injections, and generated or participated in generating all of the histological material to date. With her coursework behind her she will be free to continue to generate high-quality experimental animals and data.

During the reporting year 5 of 13 rats that received GFP control vector but not BrdU satisfied inclusion criteria to advance through stage 3f and are currently in stage 3g of the approved statement of work (SOW). These rats provide the statistical power necessary to establish to a high degree of certainty that control vector rats continue to exhibit a fully kindled state unless treated with the therapeutic SST vector. Because they did not receive BrdU, they will also permit comparison to determine whether BrdU affects kindling seizure severity or the therapeutic efficacy of AAV-SST. This will provide essential histological baselines for cytogenesis and inflammation effects of kindling and gene transfer. Histological examination demonstrates that 1) the vector selectively transduces neurons to produce sustained transgene expression, 2) neurons transduced by the vector include types that normally do not contain detectable SST (e.g. CA1 pyramidal neurons, dentate granule neurons), and 3) transduced neurons usually exhibit both SST and the GFP reporter (Figure 1), but some GFP-positive (i.e. vector-transduced) neurons do not contain detectable SST for reasons that are not clear.

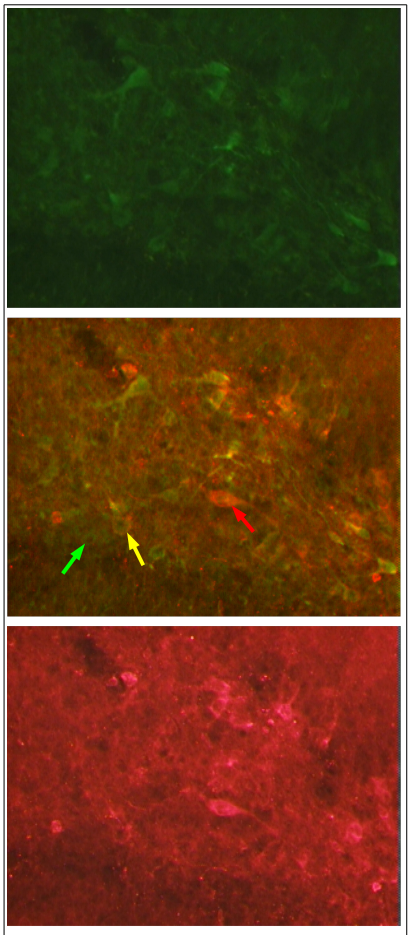

Figure 1: Overlay (center) of fluorescent reporting for GFP (green, top) and SST (red, bottom) shows that in rat dentate hilus AAV-CBA-SST-GFP delivery results in neurons immunoreactive for GFP alone (green in center overlay; no detectable SST), SST alone (red in center overlay, no GFP), or both (vector-driven SST+GFP, yellow in center image).

Four of 5 rats that received SST vector have advanced through stage 3f and are currently in SOW stage 3g. Three failed to show a therapeutic effect, although 2 of these showed histological evidence for vector expression. The remaining animal showed a total refractoriness to further elicited seizures.

Together the above animals will make it feasible to publish our demonstration that vector delivered after the emergence of a seizure-prone state can reduce subsequent seizure severity. All (N=5) rats kindled to Racine grade 5 prior to receiving GFP control vector continued to show grade 5 seizures. Of 10 rats that have now received SST vector after full kindling, 3 have shown complete elimination of seizures, 3 have shown reduction to below Racine grade 4, and 4 have shown no detectable effect.

Fifteen rats (7 control, 8 kindled) that did not receive vectors (controls) but did receive BrdU advanced through SOW stage 3e (successfully kindled), have been euthanized, and are in process for histological processing and histometry. These control group subjects will be used to test kindling effects on brain neurogenesis, in later comparisons with animals that do receive vectors. They will provide essential histological baseline data on how kindling affects cytogenesis and inflammation in the absence of the vector injection surgery.

Eight additional rats in the SST vector/BrdU early treatment condition are currently at SOW stage 3e. All of these animals are contributing baseline and experimental anatomical data that will be used to test hypotheses about vector safety and efficacy in relation to neurogenesis (Task 3) and inflammation (Task 5).

Dr. Ormerod will supervise Mr. Jeffrey Leibowitz, who joined her lab early in 2014 as a Biomedical Engineering graduate student, in conducting the BioPlex analyses of tissue samples from upcoming animals (SOW 5f). Mr. Leibowitz will evaluate neurogenesis and neuroinflammation in this project as part of his PhD dissertation. Dr. Ormerod has trained Mr Leibowitz to immunostain sections so that new cells can be quantified using stereological procedures and light microscopy, and so that the phenotypes of new cells can be verified using confocal fluorescence microscopy. She has also trained Mr. Leibowitz to conduct the microscopic analyses of neurogenesis and neuroinflammation and is currently training him to conduct the Bioplex analyses of tissue samples that will be obtained from upcoming animals.

KEY RESEARCH ACCOMPLISHMENTS:

- Administration of AAV-SST after the evolution of a seizure-prone state can reduce seizure severity in a majority of test subjects (60%), and produce a completely refractory state in a substantial fraction (30% to date).

- Based on downstream expression of GFP reporter protein, vector preproSST RNA is generated in both somatatostatinergic and non-somatostatinergic neurons.

- Immunoreactivity in principal hippocampal neurons indicates that preproSST is processed into SST14 or 28 peptide in neurons not originally somatatostatinergic. Although the abundance may be lower than in transduced neurons with inhibitory phenotypes, this suggests that diffusely distributed SST release may underlie therapeutic efficacy. Such release could be constitutive or synaptic.

- No single hippocampal subregion appears necessary or sufficient for maximum therapeutic efficacy. Efficacy has been observed in cases with expression restricted to dentate, CA3, or CA1. Conversely, as we have increased the numbers of animals tested, we have observed animals that did not show a therapeutic response despite robust expression in one or more subregion.

- Immunoreactivities for SST receptor subtypes SSTR1, 3, 4, and 5 do not show remarkable qualitative effects of kindling or SST gene transfer.

- SST receptor subtype SSTR2 shows remarkable upregulation or induction on astrocytes in response to kindling. This may indicate a mechanism involved in SST vector efficacy.

CONCLUSION: Gene delivery may provide a unique approach for localized treatment of epilepsies that are otherwise untreatable. Restoration of a seizure-free or mitigated severity condition by intracranial gene transfer could forestall or prevent the need for drastic surgical resection. Furthermore, TBI-related epilepsy tends to be more generalized and likely to involve eloquent cortical regions that fully preclude surgical options. Anti-epileptic drugs are not effective anti-epileptogenics, so SST gene transfer offers a novel approach to limiting or even reversing the clinical evolution of seizures in brain-injured military personnel and veterans. Initial findings that significant therapeutic effects occur in the majority of test subjects support further investigation of mechanisms and application parameters necessary to refine and optimize efficacy and safety.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Lay Press:

Peer-Reviewed Scientific Journals:

Invited Articles:

Abstracts: Society for Neuroscience 2014 Annual Meetings abstract control number 13114; G. Natarajan, J. McElroy, R. Zafar, J. Zhou, M. King, P. Carney, "Localized intracranial somatostatin gene delivery persistently reduces seizure severity in a rat model of temporal lobe epilepsy," Session number: 314, Session title: Non-pharmacological treatments for seizures; Monday Nov 17, 2014 8:00 am - 12:00 pm; Walter E. Washington Convention Center: Halls A-C

INVENTIONS, PATENTS AND LICENSES: Nothing to report

REPORTABLE OUTCOMES: A manuscript is in preparation describing the result that SST gene transfer initiated after the establishment of a seizure-prone state can have a therapeutic modification of seizure severity.

OTHER ACHIEVEMENTS: CoPIs King and Carney, along with 2 distinguished traumatic brain injury investigators at the at the North Florida/South Georgia VA Medical Center's Brain Rehabilitation Research Center, were awarded a Small Projects in Rehabilitation (SPiRE) grant from the VA Rehabilitation Research and Development service. This 2 year award (8/1/2014-7/31/2016) incorporates the rat kindling model of epilepsy used in the Army project with a closed-head traumatic brain injury model, to study preclinical efficacy and safety of intracranial somatatostatin gene transfer. Formal assessment of safety and functional outcome measures, and development of understanding the effects of prior brain injury on epileptogenesis and intracranial gene therapy, is specifically relevant to the translation of our approach to injured military personnel. The VA project complements but does not overlap the objectives of the Army project.

REFERENCES:

APPENDICES: SFN2014Natarajan.pdf

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NEUROSCIENCE 2014

Presentation Abstract

Program#/Poster#:	314.18/S7
Presentation Title:	Localized intracranial somatostatin gene delivery persistently reduces seizure severity in a rat model of temporal lobe epilepsy
Location:	WCC Hall A-C
Presentation time:	Monday, Nov 17, 2014, 8:00 AM -12:00 PM
Presenter at Poster:	Mon, Nov. 17, 2014, 9:00 AM - 10:00 AM
Topic:	++C.07.h. Anticonvulsant and antipileptic therapies
Authors:	***G. NATARAJAN**[1], J. MCELROY[2], R. ZAFAR[2], J. ZHOU[2], M. KING[2], P. CARNEY[2]; [1]Biomed. Engineering, Pediatrics, Neurosci., [2]Univ. of Florida, Gainesville, FL
Abstract:	The neuropeptide somatostatin (SST) is co-expressed with the inhibitory neurotransmitter gamma-amino butyric acid (GABA) in some neurons in the hippocampus and plays a major role in modulating neural excitability. Previous studies (Zafar et al., 2012) demonstrated that SST overexpression in the hippocampus significantly reduces the development of seizures during early epileptogenesis. Our current study tests whether intracranial delivery of an SST vector could similarly suppress seizures when delivered after a stable seizure state is established. We tested the putative anticonvulsant and disease-modifying properties of SST overexpression in vivo in a rat amygdala electrical kindling model of temporal lobe

epilepsy. Adult male Sprague Dawley rats (250-300g) were implanted with electrodes in the amygdala bilaterally and electrically stimulated to produce three consecutive Racine grade 5 seizures (Racine et al., 1972). Following this, an adeno-associated viral (AAV) vector mediated preprosomatostatin gene (AAV-CBa-SST-GFP) was injected bilaterally into the dentate gyrus (DG) and the cornu ammonis (CA1) subfields of the hippocampus (8 µl total). After allowing asymptotic gene expression to develop for 3 weeks without electrical stimulation, all animals were re-tested at periodic intervals under the same conditions using previously effective seizure evoking intensities. Results demonstrated that rats with no injections (kindled controls, n=2) or rats with inadvertent extrahippocampal vector delivery ascertained by the lack of GFP expression in the hippocampus (misinjection controls, n=2) continued to consistently exhibit grade five seizures upon retesting. In contrast, rats with confirmed hippocampal SST gene expression showed a reduction in seizure grade (n= 3/6 <Racine grade 4 seizures) or did not have observable seizures behaviorally and electrographically (n =2/6) with prolonged repetition and elevated stimulation intensities over the three week time period of testing. The putative anticonvulsant and disease modifying properties of intrahippocampal SST vector delivered after a seizure state has fully developed extends our previous demonstration of efficacy in preventing temporal lobe epileptogenesis (Zafar et al., 2012). Our preliminary findings suggest that localized, persistent intracranial SST supplementation could be an effective therapeutic intervention, with a specific and immediate therapeutic potential administered prior to last-resort neurosurgical resections in pharmacoresistant cases of temporal lobe epilepsy.

Disclosures: **G. Natarajan:** None. **J. McElroy:** None. **R. Zafar:** None. **J. Zhou:** None. **M. King:** None. **P. Carney:** None.

Keyword (s): somatostatin

gene therapy

temporal lobe epilepsy

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The Wilder Center of Excellence for Epilepsy Research

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